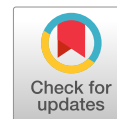


# Revista Española de Nutrición Humana y Dietética

## Spanish Journal of Human Nutrition and Dietetics

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### RESEARCH ARTICLE

## Redefining Carob-by Products as Functional Ingredients for the Future Foods

➤ Redefinición de los subproductos de algarroba como ingredientes funcionales para los alimentos del futuro

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#### KEYWORDS

*Ceratonia siliqua* L.

Fractions

Revalorization

Sustainability

Innovation

#### ➤ ABSTRACT

**Introduction:** For decades, the carob tree (*Ceratonia siliqua* L.) has been a cornerstone of Mediterranean agriculture, primarily used as livestock feed. Emerging research now highlights its remarkable nutritional and functional potential, positioning carob by-products as valuable ingredients for the next generation of functional foods.

**Methodology:** This comprehensive review examines the nutritional profile, phytochemical composition, and health-promoting properties of carob derivatives, integrating evidence from in vitro studies to assess their antioxidant, antibacterial, anticarcinogenic, and anti-inflammatory activities.

**Results:** Carob by-products are naturally rich in insoluble fiber and bioactive compounds — including phenolic compounds, inositols, and essential vitamins — with confirmed functional properties that make them promising candidates for developing health-enhancing food products. Their upcycling also aligns with circular economy and sustainability principles.

**Conclusions:** Carob emerges as a versatile, plant-based ingredient with strong potential to shape the future of functional foods. Continued research will drive innovation and reinforce its role in eco-friendly, health-promoting food systems.

## PALABRAS CLAVE

*Ceratonia siliqua* L.

Fracciones

Revalorización

Sostenibilidad

Innovación

## RESUMEN

**Introducción:** Durante décadas, el algarrobo (*Ceratonia siliqua* L.) ha sido un pilar de la agricultura mediterránea, utilizado principalmente como alimento para el ganado. Investigaciones recientes destacan su notable potencial nutricional y funcional, posicionando sus subproductos como ingredientes valiosos para la próxima generación de alimentos funcionales.

**Metodología:** Esta revisión integral analiza el perfil nutricional, la composición fitoquímica y las propiedades beneficiosas para la salud de los derivados del algarrobo, integrando evidencia procedente de estudios in vitro para evaluar sus actividades antioxidante, antibacteriana, anticancerígena y antiinflamatoria.

**Resultados:** Los subproductos del algarrobo son naturalmente ricos en fibra insoluble y compuestos bioactivos — entre ellos fenoles, inositoles y vitaminas esenciales — con propiedades funcionales confirmadas que los convierten en candidatos prometedores para el desarrollo de alimentos con beneficios para la salud. Su revalorización se alinea además con los principios de economía circular y sostenibilidad.

**Conclusiones:** La algarroba se perfila como un ingrediente versátil de origen vegetal con alto potencial para definir el futuro de los alimentos funcionales. La investigación continuada impulsará la innovación y reforzará su papel en sistemas alimentarios ecológicos y beneficiosos para la salud.

KEY  
MESSAGES

1. Underutilized carob fractions exhibit an extraordinary nutritional and phytochemical profile.
2. Carob pulp is rich in carbohydrates and fiber, while carob seeds are abundant in proteins and fats.
3. Carob fractions are rich in phenolic compounds, with gallic acid as a primary compound, and flavonoids such as quercetin as a main one
4. The high phytochemical content of carob fractions contributes to their antioxidant, anti-inflammatory, antimicrobial, and anticarcinogenic properties, among others.

## CITATION

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## INTRODUCTION

The carob tree (*Ceratonia siliqua* L.) has been cultivated since ancient times in the arid and semi-arid regions of Mediterranean countries, including Cyprus, Greece, Italy, Morocco, Portugal, Spain, and Turkey<sup>1,2</sup>. From an environmental point of view, the carob tree is an excellent crop due to its hardiness and indifference to soil nature<sup>3,4</sup>. Its leathery leaves contribute to water loss control and can absorb water effectively from the soil to compensate for atmospheric losses. Moreover, it exhibits a high capacity for carbon dioxide fixation, which makes it an effective crop for capturing CO<sub>2</sub> from the atmosphere<sup>5</sup>.

Given its low environmental footprint, carob cultivation aligns with multiple Sustainable Development Goals (SDGs) established by the Food and Agriculture Organization of the United Nations<sup>6</sup>. Specifically, it supports: (i) responsible production and consumption (SDG 12), (ii) urgent climate action (SDG 13), and (iii) the protection, restoration, and sustainable use of terrestrial ecosystems while combating land degradation (SDG 15). Despite these advantages, carob cultivation has declined over the past decade<sup>7</sup>. Concretely, global carob production fell from approximately 126,405 tons in 2012 to just 56,424 tons in 2022.

Traditional uses of this crop include animal feed and the extraction of locust bean gum (E-410) from its seeds. However, these traditional industrial applications alone have proven insufficient to ensure the economic viability of carob cultivation, leading to a noticeable reduction in its cultivation in recent years. To reverse this trend, it is crucial not only to promote awareness of carob's sustainability and climate adaptability but also to explore new strategies that enhance its profitability for farmers.

As shown in Figure 1, the carob tree consists of several fractions with potential alternative uses. It produces leaves with a tough epidermis rich in phenolic compounds, which provide protection against environmental stress. Its fruit, the pod, undergoes a maturation process in which it transitions from green to dark brown, developing an elongated structure containing both pulp (also known as kibbles) and seeds. The pulp, a fibrous and carbohydrate-rich component, is widely used as a natural sweetener and a cocoa substitute in the food industry. The seeds, on the other hand, comprise three main components: (i) the episperm (peel), which forms the external protective layer; (ii) the endosperm, a nutrient-rich tissue crucial for embryo development; and (iii) the germ, which contains the embryo and plays a key role in seed germination and propagation.

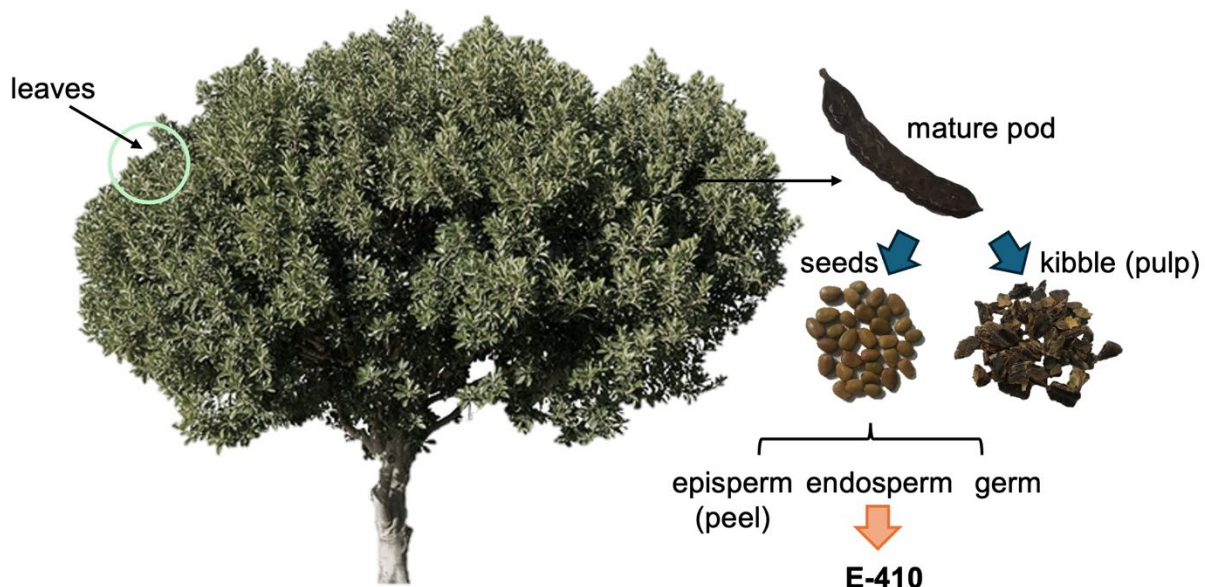
Considering the growing interest in novel, healthy, functional, and locally sourced ingredients, this comprehensive review aims to elucidate the nutritional profile, phytochemical composition, and potential health benefits of the different fractions of the carob tree, including its pulp, seeds or leaves, to explore new applications in food formulations.

## METHODOLOGY

### Scientific literature review design

A review was performed using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines<sup>8</sup> to identify the most relevant studies highlighting the nutritional value, phytochemical composition, and health benefits of carob tree and

**Figure 1.** Diagram of the different fractions of the carob tree (leaf) and its fruit (pulp and seed).

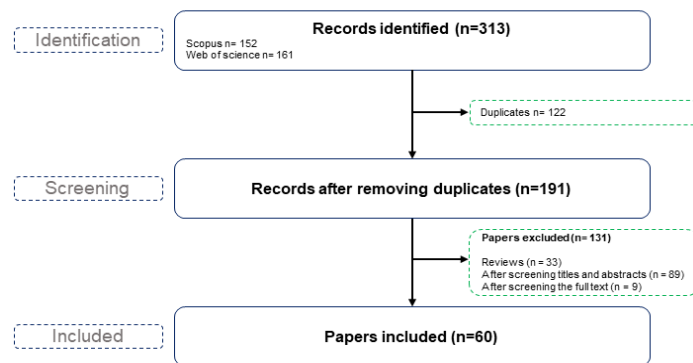


fruit fractions. Scopus and Web of Science were used as scientific browsers considering the keywords 'carob', carob fraction ('pulp', 'germ', 'peel', 'leaf', 'leaves'), nutrients ('carbohydrate', 'protein', 'fat', 'mineral', 'fiber', and 'vitamin'), 'phytochemical', 'health', and 'functional'. The date of the search covered studies from 2018 until 31<sup>st</sup> December 2024. Original research papers indexed in the JCR-SCI were selected, excluding reviews.

### Criteria for inclusion and exclusion of studies

Following the identification of relevant scientific articles, specific inclusion and exclusion criteria were applied to ensure the relevance and quality of the selected studies. Figure 2 presents the details of the records identified in this review and a summary of the selection process followed for the included research papers. Briefly, the present review encompassed articles examining various carob fractions, quantifying and identifying their nutrients and phytochemicals, as well as studies (both in vitro and in vivo) investigating their functionality and potential impact on human health. However, studies where carob fractions were used as animal feed, ingredients for food products, or adjuvants in other processes were excluded from this review.

**Figure 2.** PRISMA flow chart of the present scientific literature review.



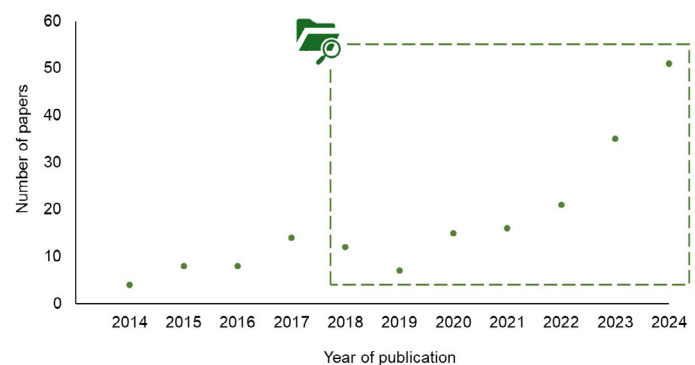
## RESULTS

The number of scientific manuscripts covered by the scientific literature review is illustrated in Figure 3. As can be seen, research on this topic has increased significantly since 2014, highlighting the growing interest in understanding the properties and potential applications of this valuable ingredient. The key findings are presented below and categorized into three sections: nutritional composition, phytochemicals, and functional activity of the different carob fractions.

## NUTRITIONAL COMPOSITION OF THE DIFFERENT CAROB FRACTIONS

As a first step, the nutritional profile of different carob fractions is elucidated to better understand their potential contributions to human health and dietary applications.

**Figure 3.** Number of scientific papers from the last ten years found in Scopus using the keywords detailed in the material and method section. The green box in dashed lines delimits the time interval in which this review is framed.



## Carbohydrates

Carbohydrates are the primary nutrients in carob and are usually expressed as total sugar concentration. As shown in Table 1 (data expressed on dry basis -d.b.), the pulp contains most of the sugars, with 32.5–65 g/100 g, while the seed only contains 5.2–9.4 g/100 g<sup>3,9–14</sup>. This high sugar content is responsible for the characteristic sweetness perception of carob<sup>14</sup>. Furthermore, the leaves of adult trees also contain a considerable proportion of sugars (15.7 g/100 g) compared to those of young trees (1.26 - 4.9 g/100 g, respectively)<sup>9</sup>. According to Boutasknit et al.<sup>15</sup> total soluble sugars content in carob leaves plays a key role in maintaining a high leaf osmotic potential.

Regarding the type of sugars, sucrose is the main sugar in the carob pod, seed and leaves with 55, 7.2 and 9.5 g/100 g, respectively. Glucose levels exceed fructose in leaves with values of 5.0 and 2.6 g/100 g of glucose and fructose, respectively, meanwhile seeds possess 0.4 g/100 g of glucose but it does not contain fructose<sup>9</sup>. Furthermore, D-pinitol is a prominent low molecular weight carbohydrate mainly found in carob pods with concentrations ranging from approximately 2 to 12 g/100 g, depending maturity and processing conditions. It plays a role in the adaptation mechanism to stress conditions such as high salinity or water deficit<sup>16</sup>. In addition, D-pinitol has been shown to regulate blood insulin levels and to exhibit insulin-mimetic and anti-diabetic properties<sup>17</sup>.

Therefore, carob by-products can be a good option as a natural sweetener alternative because they have a significant amount of sugar, but being their glycemic index so low due to, among other factors, to the presence of D-pinitol.

## Proteins

The carob pod, composed of both pulp and seeds, contains between 3.73 and 6.95 g/100 g of protein<sup>9,18</sup>. The pulp, which makes up the majority of the pod, has a protein content ranging from 2.66 to 6.48 g/100 g<sup>9–13,19,20</sup>. In contrast, the seed fraction contains significantly more protein (15.54 – 26.64 g /100 g), with the germ portion reaching 49.9 – 53.13 g/100 g<sup>21–23</sup>. No data is available on the protein content of the carob seed episperm during the studied period. According to Mahtout et al.<sup>9</sup>, the carob leaf fraction has  $7.4 \pm 0.1$  g/100 g protein. However, in younger plants, such as seedlings, protein levels are considerably lower, ranging from 0.78 to 1.34 g/100 g<sup>15,24</sup>.

From a nutritional perspective, carob seed protein is particularly valuable due to its complete amino acid profile, which includes all essential amino acids<sup>21</sup>. The predominance of aliphatic amino acids—such as isoleucine, leucine, alanine, and valine—enhances its overall nutritional quality<sup>14</sup>. However, the high proportion of insoluble proteins, especially in the seed germ, may result in lower digestibility compared to the pulp and leaf fractions<sup>9</sup>. Despite this, the protein-rich nature of carob seed germ surpasses that of common plant-based protein sources such as soybean flour (40 g/100 g), chickpeas (25 g/100 g), and rice (7.5 g/100 g)<sup>25–27</sup>. In addition to the high nutritional value, carob seed germ contains caroubin, a protein fraction with gluten-like properties, which presents a valuable opportunity for the food industry, particularly in gluten-free product development<sup>22</sup>. The predominance of soluble proteins in carob seed germ enhances its functional versatility, facilitating its incorporation into various food applications. Given its polymeric protein composition, carob protein would contribute to desirable textural properties of gluten-free products, offering a viable alternative for individuals with gluten intolerance while maintaining the structural integrity of food products.

## Fats

The lipid content in carob fractions is generally low. The highest fat concentration is found in carob seeds (2.0–2.6 g/100g), particularly in the carob germ (2.92–7.1g/100g)<sup>28</sup>. In contrast, the fat content in carob pulp ranges from 0.10g/100g to 2.38 g/100g<sup>10</sup>. Regarding fat composition, Amar et al.<sup>29</sup> reported that carob seeds contain 62.7g/100 g unsaturated fatty acids and 38.3 g/100g saturated fatty acids, indicating a well-balanced profile that likely contributes to a solid texture at room temperature. This property is advantageous for food formulations, as it enhances structural stability without requiring hydrogenation, a process associated with the formation of trans fats and their associated health risks.

Among the unsaturated fatty acids, oleic acid (37.1 g/100 g), linoleic acid (12 g/100 g), and palmitoleic acid (2.4 g/100 g) are the most abundant in carob pulp, suggesting potential health benefits. Oleic acid is known to support cardiovascular health by improving cholesterol levels, while linoleic acid plays a crucial role in maintaining cell membrane integrity and regulating inflammation. Palmitoleic acid contributes to metabolic health and exhibits anti-inflammatory properties. Regarding saturated fatty acids, palmitic acid (17.9 g/100 g) is the predominant type in carob pods, playing a role in immune function and gene regulation through its influence on signaling pathways<sup>30</sup>. Similar fatty acid distribution patterns have been observed in carob pods<sup>31</sup>.

## Fiber

Fiber refers to indigestible polysaccharides such as hemicellulose, cellulose, and lignin. Among the studies reviewed, [Table 1](#) shows that carob pods, comprising both pulp and seed fractions, are recognized for their high fiber content although there were differences among studies as values ranged from 3.11 g/100 g<sup>13</sup> to 4.9–7.7 g/100 g<sup>18</sup>. From a nutritional and functional perspective, carob fiber contributes to digestive health by promoting gut motility, supporting both the growth of beneficial gut microbiota and cholesterol regulation. The high fiber content of carob also plays a role in glycemic control by slowing glucose absorption, which may be beneficial for individuals with diabetes.

## Minerals

The mineral composition depends on the carob fraction analyzed, as well as on its genotype, cultivar, geographical origin, climatic conditions, harvesting stage or the age of the tree, soil conditions, and the season of the year<sup>12,32</sup>.

Potassium is the predominant mineral in carob pulp, with reported concentrations ranging from 1121.1 to 1300.2 mg/100g, followed by calcium (164–451mg/100g), phosphorus (41.9–57.2mg/100g), and magnesium (40.4 – 186.9mg/100g)<sup>12,32–34</sup>. In carob seeds, sodium is present in the highest concentration (3590–4030mg/100g), followed by calcium (217.4–381.96mg/100g)<sup>33</sup>. According to the results, different carob by-products would offer significant nutritional benefits, with potassium supporting cardiovascular health, calcium promoting bone strength and muscle function, phosphorus aiding energy metabolism, and magnesium contributing to nerve function and metabolic regulation<sup>12,32,33</sup>.

## Vitamins

Vitamin C, quantified as ascorbic acid, has been reported to be present in carob pods and leaves with values ranging from 5.8–10.2 and 3 – 52 mg/100 g, respectively ([Table 1](#)). In this case, it suggests potential antioxidant benefits, contributing to immune function, collagen synthesis, and protection against oxidative stress<sup>18,35</sup>. As

Table 1 shown, carob pulp and seeds also contain vitamin E, an essential fat-soluble antioxidant that protects cells from oxidative damage and supports immune function and skin health. The presence of  $\alpha$ -tocopherol, the biologically active form, along with  $\beta$ -,  $\gamma$ -, and  $\delta$ -tocopherols, suggests that carob may contribute to overall antioxidant defense and lipid stability in the diet. Likewise, the presence of  $\beta$ -carotene suggests that carob could serve as a potential source of vitamin A, as  $\beta$ -carotene is a provitamin A compound that the body can convert into its active form<sup>28,29,36</sup>.

## PHYTOCHEMICALS OF THE DIFFERENT CAROB FRACTIONS

Phytochemicals are associated with a wide range of biological and functional properties, including antioxidant, antimicrobial, anti-allergic, anti-inflammatory, anti-carcinogenic, and antiviral activities<sup>35</sup>. Table 2 presents the phytochemical composition of each carob fraction. Among the studies reviewed, polyphenols and terpenoids are the most commonly identified phytochemicals in carob.

### Polyphenols

Polyphenols are bioactive compounds characterized by the presence of one or more phenolic rings. These compounds, present in significant quantities, are known for their beneficial effects against oxidative stress. Based on their chemical structure, including the number and arrangement of these rings, polyphenols can be classified into phenolic acids, flavonoids, stilbenes, coumarins, and tannins<sup>42</sup>. As shown in Table 2, phenolic acids are the predominant polyphenols in carob pulp and seeds, whereas flavonoids are more abundant in carob leaves<sup>43-46</sup>. Additionally, carob fractions contain considerable quantities of tannins.

Comparison among data from different sources allows observing significant variations between different carob fractions and within extracts of the same fraction. Authors suggest that these discrepancies might be attributed to several factors, including geographical origin, ripening stage, plant fraction analyzed, and the extraction techniques used prior to analysis<sup>43,47,48</sup>.

Beyond assessing total phenolic, flavonoid, and tannin content across carob fractions, many studies focus on isolating and identifying specific polyphenols, given their distinct functional properties. A review of selected studies has identified over 26 different polyphenolic compounds in various carob fractions<sup>3,13,33,35,37,42-46,49-52</sup>. Regarding phenolic acids, Table 2 shows that gallic acid (205.10-2500 mg/kg) is the most abundant in carob, followed by syringic acid (1360 mg/kg), chlorogenic acid (135.44-260 mg/kg), caffeic acid (164.20 mg/kg), 2-hydroxycinnamic acid (80.31 mg/kg), gentisic acid (31.03-190.85 mg/kg), and ferulic acid (9.18-13 mg/kg)<sup>13,37,42,49,53,54</sup>. The presence of these compounds is very relevant, since these phenolic acids are bioactive compounds with significant functional relevance due to their antioxidant, anti-inflammatory,

and potential health-promoting properties. These compounds contribute to the neutralization of free radicals, thereby reducing oxidative stress and supporting cellular protection. Additionally, their presence in the diet has been associated with potential benefits in cardiovascular health, metabolic regulation, and neuroprotection. The ability of phenolic acids to modulate gut microbiota composition further enhances their role in digestive health, highlighting their importance as functional dietary component<sup>55</sup>.

Flavonoids are polyphenolic secondary metabolites classified into subclasses such as flavones, flavonols, flavanones, catechins, and isoflavonoids based on their structural characteristics. The flavones identified in carob extracts are luteolin (80-90 mg/kg), apigenin (50-696.12 mg/kg), and flavone (62.3 mg/kg). The flavonols included kaempferol-O-hexoside (160-180 mg/kg), kaempferol rutinoside (10 mg/kg), kaempferol-O-pentoside (30-60 mg/kg), isorhamnetin (3.62-5.67 mg/kg), rutin (60-100 mg/kg), isoquercetin (264.79-817.61 mg/kg), quercetin pentoside (4.4-5.5 mg/kg), quercetin rhamnoside (57.3-61.7 mg/kg), and myricetin (8000.66-9000.57 mg/kg). The detected catechins are epigallocatechin gallate (960-1390 mg/kg), epicatechin (40-70 mg/kg), epicatechin gallate (360-460 mg/kg), and catechin (30-50 mg/kg). Lastly, naringenin (96.5-362.5 mg/kg) is the only flavanone isolated. From a functional point of view, flavonoids possess strong antioxidant and anti-inflammatory properties, contributing to cardiovascular health by improving endothelial function and lipid metabolism. Additionally, flavonoids support cognitive function, reduce the risk of neurodegenerative diseases, and modulate gut microbiota, enhancing metabolic and immune regulation<sup>55</sup>.

Finally, tannins are polyphenolic compounds with multiple hydroxyl groups (-OH) that allow them to interact with proteins and other macromolecules. Of all the tannins found in nature, ellagic acid (398.85-940.53 mg/kg)<sup>42</sup> and proanthocyanidins (0.1-11 mg/kg)<sup>35</sup> are identified and quantified in carob fruit and leaves, respectively. These molecules contribute to the astringency of food products<sup>56</sup>. Several studies suggest that tannins might reduce oxidative stress, supporting gut health, and even lowering the risk of chronic diseases such as cardiovascular disorders and certain cancers. Furthermore, their ability to bind to dietary iron can help regulate iron absorption, which may be beneficial for individuals with iron overload conditions<sup>35</sup>.

### Terpenoids

In addition to polyphenols, recent research has identified and quantified two distinct groups of terpenoids in carob pods and seeds<sup>33</sup>. The first group consists of carotenoids, essential pigments involved in photosynthesis and plant coloration. Among these,  $\beta$ -carotene and lycopene have been quantified at concentrations of 28 mg/kg and 38.92 mg/kg in carob pods, and 327 mg/kg and 198.82 mg/kg in carob seeds, respectively. Additionally, the xanthophyll zeaxanthin has been detected at concentrations of 38.17 mg/kg in carob pods and 207.35 mg/kg in seeds.

**Table 1.** Nutritional composition of the different carob fractions.

Carob fraction Nutrients (g/100 g)	Pod		Seed		Leaves	Ref.
	Pulp	Seed	Germ	Peel		
<b>Carbohydrates* (g/100 g)</b>	49.36 – 69.36		49.26	77.88	15.7	9–14,18,37,38
	32.5 – 65	5.2 – 9.4				
<b>Proteins (g/100 g)</b>	3.73 – 6.95		49.9 – 53.13	5.81	7.4	9–13,18–23,25,37,39,40
	2.66 – 6.48	15.54 – 26.64				
<b>Fats (g/100 g)</b>	0.18 – 2.4		2.92 – 7.1	0.34	4.5	9–11,13,19,28,29,31,37,40
	0.10 – 2.38	2.0 – 2.57				
<b>Fiber (g/100 g)</b>	52.35		57.7	60.26	n.a.	10,13,18–20,25,37,39,40
	24.37 – 79.23	43.46 – 65.61				
<b>Minerals (mg/100 g)</b>	n.a.		n.a.	n.a.	N: < 1400 K: < 1000 P: < 250 Mg: < 250 Ca: < 250	11,12,32–34,41
	K: 353.6 – 1300.2 N: 530 – 620 Ca: 164 – 451.15 Cl: 198.2 Mg: 40.4 – 186.9 P: 41.9 – 314.6 Na: 13.9 – 81.7 Fe: 5.26 – 8.29 Zn: 1.80 – 2.61 Mn: 0.6 Cu: 0.42 – 0.56 PO <sub>4</sub> : 121.3 SO <sub>4</sub> : 50.5 NH <sub>4</sub> : 14.7 NO <sub>3</sub> : 6.6	K: n.a. N: 3590 – 4030 Ca: 217.4 – 381.96 Cl: n.a. Mg: 104.3 – 149.1 P: n.a. Na: n.a. Fe: 3.49 – 6.46 Zn: n.a. Mn: n.a. Cu: n.a. PO <sub>4</sub> : n.a. SO <sub>4</sub> : n.a. NH <sub>4</sub> : n.a. NO <sub>3</sub> : n.a.				
<b>Vitamin C (mg/100 g)</b>	5.8 – 10.2		n.a.	n.a.	3 – 52	18,35
	n.a.	n.a.				
<b>Vitamin E (mg/100 g)</b>	n.a.		<b>α-tocopherol:</b> 1.48 <b>β+γ-tocopherol:</b> 3.68	n.a.	n.a.	28,29,31
	Vit. E: 0.33 – 2.68 <b>α-tocopherol:</b> 4.31 – 8.77 <b>γ-tocopherol:</b> 10.37 – 27.78 <b>δ-tocopherol:</b> 1.41 – 5.23	Vit. E: n.a. <b>α-tocopherol:</b> 2.86 – 7.59 <b>γ-tocopherol:</b> 4.59 – 22.86 <b>δ-tocopherol:</b> 0.88 – 2.62				

\*carbohydrate content has been expressed as sugar content; n.a.: means that no data were found in the scientific studies evaluated.

The second group comprises chlorophylls, the green pigments essential for photosynthesis. Chlorophyll a concentrations were measured at 7.58 mg/kg in carob pods and 2.83 mg/kg in seeds, while chlorophyll b concentrations were 4.87 mg/kg in pods and 3.59 mg/kg in seeds.

## FUNCTIONAL ACTIVITY OF THE DIFFERENT CAROB FRACTIONS

With growing consumer interest in plant-based ingredients, there is increasing recognition that carob not only provides essential nutrients but also bioactive compounds with significant health benefits. As already commented in previous sections, these phytochemicals contribute to antioxidant, anti-inflammatory, antimicrobial, anticarcinogenic, and antidiabetic properties of carob, making it a promising functional ingredient for disease prevention and health promotion. Thus, after identifying the nutritional profile and phytochemical composition of the carob fractions, including the pod, pulp, peel, germ, and leaves, their functional properties have been extensively reviewed.

### Antioxidant activity

Among the functional properties of carob, antioxidant activity is one of the most extensively studied. Table 3 provides an overview of studies evaluating the antioxidant activity of various carob fractions using different *in vitro* spectrophotometric assays, including DPPH, FRAP, ABTS, and ORAC, with results expressed in g Trolox E/100 g. Carob leaves exhibited the highest antioxidant capacity with values ranging from 12 to 179 g Trolox E/100 g<sup>56,57</sup>. In contrast, carob pulp demonstrated moderate antioxidant activity across different methods, with reported values ranging from 1.44 to 12.22 g Trolox E/100 g, depending on the study<sup>51,55</sup>. Another study reported approximately 7 g Trolox E/100 g for carob seeds and germ and around 4.5 g Trolox E/100 g for carob pod<sup>37</sup>.

The high antioxidant capacity observed in carob by-products highlights their potential to combat oxidative stress, an imbalance between free radicals and antioxidants that contributes to the onset of chronic diseases such as cardiovascular disorders, cancer, and neurodegenerative conditions<sup>56,57</sup>. This makes carob fractions promising candidates for functional food applications with potential therapeutic benefits. However, it is important to note that most of the current evidence is based on *in vitro* assays, and further *in vivo* and clinical studies are needed to confirm these health effects in humans. In addition, the variability in reported antioxidant activity is largely influenced by differences in extraction methods, solvent types, and sample characteristics<sup>58,59</sup>. Among these factors, maturation stage had the most significant impact, as unripe carob products are rich in phenolic compounds and

acids, which gradually converted into sugars during maturation<sup>60</sup>.

### Anti-inflammatory activity

The anti-inflammatory potential of carob by-products and extracts (germ, seed peel, pod, and pulp) has also been studied through *in vitro* experiments<sup>37,49</sup>. Results evidenced that carob pod and germ extracts produce a reduction of two molecules involved in inflammation and immune responses: pro-inflammatory cytokine (TNF- $\alpha$ ) and prostaglandin D2 (PGD<sub>2</sub>), probably due to the presence of flavonoids and phenolic compounds<sup>37</sup>.

Furthermore, an *in vivo* study conducted in mice evaluated the neuroprotective effect of carob leaf extracts in i) reducing harmful proteins linked to Alzheimer disease such as myloid- $\beta$ 42 (A $\beta$ 42) and p-Tau, ii) preserving neurotransmitters as acetylcholine, iii) in blocking major triggers of brain inflammation as NF- $\kappa$ Bp65 and TNF- $\alpha$ , iv) protecting the brain cells as Nrf2 and HO-1 against oxidative stress and damage, and v) stabilizing  $\beta$ -catenin, molecule responsible for cell survival and brain function, thus providing preclinical evidence of carob's potential in neurodegenerative disease prevention<sup>44</sup>.

### Anticarcinogenic activity

Reactive oxygen species (ROS) play a dual role in cellular physiology, acting as essential signaling molecules or, when excessively accumulated, exerting toxic effects. It is well established that prolonged ROS accumulation—whether from endogenous or exogenous sources—induces cellular senescence and apoptosis. Apoptosis, in turn, is closely linked to the dysregulation of intracellular redox signaling pathways involved in carcinogenesis.

To counteract carcinogenic pathways and molecules, several *in vitro* studies have investigated the potential anticancer effects of carob fractions on human cells. In this sense, Ben Ayache et al.<sup>58</sup> demonstrated that carob extracts had a significant phenotypic proapoptotic capability on colon cancer cell lines, such as THP1, MCF-7, and LoVo human cells. Moreover, amongst the different carob extracts evaluated, the seeds presented the highest anticarcinogenic activity on the targeted human cell carcinomas. Similarly, Elbouzidi et al.<sup>45</sup> investigated the cytotoxic effect of carob leaves against breast cancer (MCF-7) and two metastatic adenocarcinoma lines (MDA-MB-231 and MDA-MB-436) with the MTT assay. The results observed in this work indicated that carob extracts had low cytotoxicity against the three cell lines, with the MCF-7 being the most sensitive. Furthermore, the carob extract did not exhibit cytotoxicity towards normal cells, suggesting that carob extracts have a targeted and low toxic effect on cancer cells. Although these findings provide promising preliminary evidence, further *in vivo* and clinical studies would be needed to confirm their therapeutic potential.

**Table 2.** Phytochemical composition of the different carob fractions.

Phytochemical (mg/kg dw)	Carob fraction		Seed		Leaves	Ref.
	Pulp	Seed	Germ	Peel		
<b>Phenolic acids</b>						
<b>3,4-Dihydrobenzoic acid hexoside</b>	n.a.		n.a.	n.a.	0-10	35
	n.a.	n.a.				
<b>5-caffeoylquinic acid</b>	n.a.		n.a.	n.a.	n.a.	19
	n.d - 12.23	n.a.				
<b>Caffeic acid</b>	n.a.		n.a.	n.a.	20-760	19,35,43,51
	n.d.-331.37	n.a.				
<b>Chlorogenic acid</b>	n.a.		n.a.	n.a.	10-40	13,35,43,51
	n.d.-260	n.a.				
<b>Cinnamic acid</b>	n.a.		n.a.	n.a.	n.a.	43,51
	n.d.-9.74	n.a.				
<b>Ferulic acid</b>	n.a.		n.a.	n.a.	10-30	35,43
	n.d.-9.18	n.a.				
<b>Gallic acid</b>	1518.38		213.78	137.90	6610-9090	13,19,35,37,43,51
	183.92-2525.08	n.a.				
<b>Gentisic acid</b>	n.a.		n.a.	n.a.	n.a.	35,43
	31.03 -190.85	n.a.				
<b>p-coumaric acid</b>	n.a.		n.a.	n.a.	230-400	19,35,51
	n.d.-79.34	n.a.				
<b>p-hydroxybenzoic acid</b>	n.a.		n.a.	n.a.	410-440	35
	n.a.	n.a.				
<b>Protocatehuic acid</b>	n.a.		n.a.	n.a.	70-80	35
	n.a.	n.a.				
<b>Rosmarinic acid</b>	n.a.		n.a.	n.a.	10-20	35,51
	n.d.	n.a.				
<b>Sinapic acid</b>	n.a.		n.a.	n.a.	n.a.	19
	n.d.- 20.3	n.a.				
<b>Syringic acid</b>	n.a.		n.a.	n.a.	40-60	13,19,35
	33.43-1360	n.a.				
<b>Flavonoids</b>						
<b>C-hexoside-O-rhamnosyl-hexoside apigenin</b>	n.d.		258.76	189.43	n.a.	37
	n.a.	n.a.				
<b>Apigenin</b>	n.a.		n.a.	n.a.	50	19,35
	194.66-696.12	n.a.				
<b>Catechin</b>	n.a.		n.a.	n.a.	30-50	35,42,43
	n.d-171.05	n.a.				
<b>Crismaritin</b>	n.a.		n.a.	n.a.	n.a.	42
	n.d.-30.81	n.a.				

Phytochemical (mg/kg dw)	Carob fraction		Seed		Leaves	Ref.
	Pulp	Seed	Germ	Peel		
Epicatechin	n.a.		n.a.	n.a.	40-70	35
	n.a.	n.a.				
Epicatechin gallate	n.a.		n.a.	n.a.	360-460	35,42
	29.63-79.31	n.a.				
Epigallocatechin gallate	n.a.		n.a.	n.a.	960-1390	35,42
	n.d.-19.25	n.a.				
Isoquercetrin	n.a.		n.a.	n.a.	n.a.	42
	264.79-817.61	n.a.				
Isorhamnetin	n.a.		n.a.	n.a.	n.a.	13
	5670	n.a.				
Isorhamnetin 3-O-hexoside	n.d.		88.30	107.79	n.a.	37
	n.a.	n.a.				
Isorhamnetin hexoside	n.a.		n.a.	n.a.	290-320	35
	n.a.	n.a.				
Isorhamnetin O-rhamnoside	n.d.		136.35	86.45	n.a.	37
	n.a.	n.a.				
Kaempferol	n.a.		n.a.	n.a.	n.a.	13,42
	98.37-2320	n.a.				
Kaempferol-O-hexoside	n.a.		n.a.	n.a.	160-180	35
	n.a.	n.a.				
Kaempferol-O-pentoside	n.d.		3.21	n.a.	30-60	35,37
	n.a.	n.a.				
Kaempferol O-rhamnoside	n.d.		78.90	115.02	n.a.	37
	n.a.	n.a.				
Kaempferol rutinoside	n.a.		n.a.	n.a.	n.d. - 10	35,42
	n.d.-13.66	n.a.				
Luteolin	n.a.		n.a.	n.a.	80-90	42
	7.89-44.14	n.a.				
Luteolin-7-O-glucoside	n.a.		n.a.	n.a.	n.a.	42
	n.d.-36.2	n.a.				
Myricetin	n.a.		n.a.	n.a.	8660-9570	35,43
	n.d.-60.80	n.a.				
Myricetin O-hexoside	44.18		n.d.	n.d.	n.a.	37
	n.a.	n.a.				
Naringenin	n.a.		n.a.	n.a.	n.a.	42,43
	n.d.-362.48	n.a.				
O-hexosyl-6-C-hexosyl-8-C-pentoside apigenin	n.d.		23.22 – 464.13	n.d.	n.a.	37
	n.a.	n.a.				
Quercetin	n.a.		n.a.	n.a.	n.a.	13,43,51
	n.q.-3210	n.a.				

Phytochemical (mg/kg dw)	Carob fraction		Seed		Leaves	Ref.
	Pulp	Seed	Germ	Peel		
Quercetin hexoside	n.a.		n.a.	n.a.	740-900	35
	n.a.	n.a.				
Quercetin glucoside	5.83 - 54.10		129.36	119.94 – 293.89	440-500	35,37
	n.a.	n.a.	– 382.78			
Quercetin rhamnoside	63.19		2167.31	2669.06	5730-6170	35,37
	n.a.	n.a.				
Quercitrin	n.a.		n.a.	n.a.	n.a.	51
	74.93 – 118.73	n.a.				
Rutin	n.a.		n.a.	n.a.	60-100	35,51
	94.91 – 171.11	n.a.				
<b>Tannins</b>						
Digalloyl-glucose	n.d. - 1218.50		404.14	162.21 - 85.18	n.a.	37
	n.a.	n.a.				
Galloyl-glucose derivative	n.d.		583.24	106.09	n.a.	37
	n.a.	n.a.				
Tetragalloyl-glucose	124.51		221.33	n.a.	n.a.	37
	n.a.	n.a.				
Trigalloyl-glucose	n.d.		224.98	449.41	n.a.	37
	n.a.	n.a.				
<b>Other phenolic compounds</b>						
Catechol	n.a.		n.a.	n.a.	n.a.	42,43
	n.d.-33.86	n.a.				
Ellagic acid	n.a.		n.a.	n.a.	n.a.	42
	398.85 - 940.53	n.a.				
Procyandinin trimer type B	n.a.		n.a.	n.a.	10-110	35
	n.a.	n.a.				
Resorcinol	n.a.		n.a.	n.a.	n.a.	42
	32.42-98.34	n.a.				
<b>Terpenoids</b>						
β carotene	n.a.		n.a.	n.a.	n.a.	33
	26.48 – 137.24	2.79 – 11.61				
Chlorophyll a	n.a.		n.a.	n.a.	n.a.	33
	2.75 – 7.58	1.39 – 5.07				
Chlorophyll b	n.a.		n.a.	n.a.	n.a.	33
	3.47 – 4.87	1.16 – 6.08				
Lycopene	n.a.		n.a.	n.a.	n.a.	33
	38.74 – 189.97	4.58 – 15.69				

Phytochemical (mg/kg dw)	Carob fraction				Leaves	Ref.
	Pod		Seed			
	Pulp	Seed	Germ	Peel		
Zeaxanthin	n.a.	4.21 – 14.57	n.a.	n.a.	n.a.	33

Results are expressed in mg/kg dry product. n.d. means not detectable. n.q. means not quantifiable. n.a.: means that no data were found in the scientific studies evaluated.

### Antidiabetic activity

When studying the antidiabetic properties of carob extracts, one widely researched approach is evaluating their ability to inhibit  $\alpha$ -amylase and  $\alpha$ -glucosidase. The digestive enzyme  $\alpha$ -amylase converts dietary starch into glucose before it is absorbed. Inhibition of this enzyme could help reduce elevated blood sugar levels after meals in diabetic patients. With this regard, Darwish et al.<sup>61</sup> indicated a reduction of ca. 90 % of  $\alpha$ -amylase activity in *in vitro* assays when testing extracts of carob pod. Similarly,  $\alpha$ -glucosidase, another digestive enzyme, breaks down disaccharides into glucose. Substances that inhibit  $\alpha$ -glucosidase can slow down carbohydrate absorption and help control post-meal blood sugar spikes, making them valuable in managing diabetes. In this scenario, Christou et al.<sup>62</sup> reported, through *in vitro* analysis, that carob leaf have  $\alpha$ -glucosidase and  $\alpha$ -amylase inhibitory activities of over 95% and 93%, respectively, due to D-pinitol content, among others. Moreover, gallic acid, also found in carob extracts, is considered an  $\alpha$ -glycosidase inhibitor, whose activity in managing type 2 diabetes is comparable to acarbose, an oral medication used for the treatment of type 2 diabetes. Apart from that, the enhanced antidiabetic effectiveness of carob can be attributed to its potent antioxidant properties. With this regard, D- pinitol would be able to protect pancreatic  $\beta$ -cells, which produce insulin, from oxidative damage. Furthermore, flavonoids present in carob have demonstrated the ability to boost insulin production, reduce insulin resistance, and inhibit the activity of hormone-sensitive lipase<sup>63,64</sup>. Beyond *in vitro* results, Hussein et al.<sup>65</sup> provided *in vivo* confirmation by reporting a significant reduction in blood glucose levels in diabetic rats following the administration of carob extracts, thereby reinforcing the potential antidiabetic efficacy of carob in a physiological context.

### Activity against overweight and obesity

Adipocyte hyperplasia, hypertrophy, and apoptosis are fundamental cellular processes that regulate adipose tissue expansion, a key factor in the development of overweight and obesity. In adulthood, adipogenesis (the formation of new adipocytes) plays a relatively minor role, whereas the enlargement of existing adipocytes primarily contributes to increased fat storage and adipose tissue accumulation. With this regard, Rico et al.<sup>37</sup> *in vitro* demonstrated the reduction in fat accumulation, or triacyl glyceride production, induced by seed peel and pod extract obtained from carob on

mature adipocytes at a dose of 0.1 mg/mL. This fact would indicate that these by-products are promising candidates with therapeutic potential for the prevention and/or treatment of metabolic syndrome. In another study, Martínez-Villaluenga et al.<sup>66</sup> conducted *in vitro* experiment to test the effects of carob pod extracts on 3T3-L1 mature adipocytes, demonstrating a delipidizing effect and a 32% reduction in intracellular triacylglycerol content. These results suggest a potential role of carob extracts in modulating adipocyte lipid storage, which might contribute to anti-obesity effects. In a further study, Fujita et al.<sup>67</sup> observed *in vivo* that the intake of carob pod polyphenols by mice was associated with reductions in body weight, retroabdominal fat weight, fatty liver, liver triacylglyceride levels, adipocyte hypertrophy, as well as macrophage infiltration in the adipose tissue. In addition, carob pod polyphenols inhibited adipocyte differentiation. The similar findings from both *in vitro* and *in vivo* studies strengthens the scientific evidence supporting the anti-obesity potential of carob extracts, highlighting their capacity to modulate lipid metabolism, reduce adipose tissue expansion, and attenuate obesity-related inflammation.

### Antimicrobial activity

Carob by-products also exhibit significant antimicrobial properties, primarily attributed to the presence of reactive functional groups, such as aldehydes and hydroxyls, in their phytochemical composition<sup>49</sup>. In this scenario, Ben Othmen et al.<sup>59</sup> conducted *in vitro* evaluations of the antimicrobial effects of carob leaves against three gram-negative bacterial strains (*Vibrio harveyi*, *Vibrio anguillarum*, and *Photobacterium damsela*). Their findings revealed that extracts exhibited a 60% bacterial reduction at a concentration of 0.5 mg/mL. Zahorec et al.<sup>51</sup> evaluated the antimicrobial effect of carob pulp extracts against bacteria, fungi, and yeasts. The results indicated a reduction of up to half the minimum inhibitory concentration (MIC), making promising to reduce microorganism. Likewise, El-Haddad et al.<sup>49</sup> evaluated the susceptibility of carob pulp and seeds extracts in different microorganisms (bacteria, fungi, and yeasts). The study indicated that gram-positive bacteria with MIC values ranging from 12 to 50 mg/mL were the most susceptible to the treatments followed by gram-negative strains, fungi, and yeasts with MIC values > 50 mg/mL. On other hand, the pulp showed higher antimicrobial activity than seeds. Finally, Chaalal et al.<sup>68</sup> investigated the impact of the digestive process on the antimicrobial activity of carob extracts against *Escherichia coli*,

*Enterococcus faecalis*, *Staphylococcus aureus*, *Enterobacter sakazakii*, and *Listeria monocytogenes*. Their findings revealed no significant reduction in antimicrobial activity after digestion. This is of great interest, as it suggests that the bioactive compounds responsible for this effect remain stable throughout digestion, ensuring their efficacy upon consumption. The preservation of antimicrobial activity post-digestion would enhance the potential of carob extracts as natural antimicrobial agents in functional foods and therapeutic applications, although clinical trials are needed.

### Other activities

In addition to the previously discussed properties, carob fractions have demonstrated two additional biological activities. On the one hand, Saci et al.<sup>48</sup> demonstrated *in vitro* that carob pod was able to inhibit two enzymes involved in the formation of molecules that trigger Alzheimer's disease by analyzing different stages of maturation of the carob pod. In the study, the highest IC<sub>50</sub> values for acetylcholinesterase and butyrylcholinesterase with 13.19 and 13.26 µg/mL were reached in immature pods. Consistent with the above-mentioned findings, El Sayed et al.<sup>44</sup> found in an *in vivo* assay with mice that carob leaves were able to reduce amyloid-β (Aβ) ( $p < 0.001$ ), molecules responsible for Alzheimer's disease, and change different parameters related to spatial memory. *In vitro* and *in vivo* results reinforce the scientific evidence supporting the neuroprotective potential of carob fractions, although further clinical studies are needed to confirm these effects in humans. On the other hand, Moubtakir et al.<sup>60</sup> tested *in vivo* the antiulcer activity of carob pulp extract using 250 and 500 mg/kg on Wistar rats and Swiss mice. Results showed that both concentrations

reduced approximately a 90 % ulceration index, achieving similar levels to 30 mg/kg of omeprazole, the most popular gastroprotective drug.

### CONCLUSIONS

Carob and its extracts stand out as promising ingredients for the development of future foods, thanks to their exceptional nutritional profile, functional properties, and sustainable cultivation. Its high content of low-glycemic carbohydrates, particularly D-pinitol, makes it a natural sweetener with potential benefits for blood glucose regulation. Additionally, its high fiber content supports digestive and metabolic health, while its supply of complete proteins and essential minerals further enhances its nutritional value. From a functional perspective, the presence of bioactive compounds such as polyphenols and antioxidants has demonstrated protective effects against metabolic, cardiovascular, and neurodegenerative diseases.

Given these properties, carob can be considered an exceptional for the development of next-generation functional foods, including high-protein gluten-free plant-based snacks, gut-health-enhancing prebiotic formulations, sugar alternatives for diabetic-friendly products or functional products able to contribute to a healthy ageing. Its versatility and nutritional benefits further underscore its growing recognition as a valuable food ingredient.

However, despite these promising applications, further research is needed to standardize extraction protocols and conduct clinical studies that validate carob's therapeutic potential. Addressing these gaps will ensure its broader adoption in the development

**Table 3.** Antioxidant activity of the different carob fractions, assessed using DPPH (2,2-diphenyl-1-picrylhydrazyl radical scavenging), ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation decolorization), FRAP (ferric reducing antioxidant power), and ORAC (oxygen radical absorbance capacity) assays.

Methodology	Pod		Seed		Leaves	Ref.
	Pulp	Seed	Germ	Peel		
DPPH	n.a.	n.a.	n.a.	n.a.	12 -179	51,55-57
	1.64 - 12.22	n.a.				
ABTS	n.a.	n.a.	n.a.	n.a.	n.a.	51
	1.44 - 2.75	n.a.				
FRAP	n.a.	n.a.	0.025	0.034	n.a.	13,37,51
	0.035-1.56	n.a.				
ORAC	n.a.	n.a.	7.13	7.78	n.a.	37
	4.82	n.a.				

Results are expressed in g Trolox E/100 g dry product. In order to compare the antioxidant activity among carob by-products object of this study and methodology, only the studies which have shown the antioxidant activity in w (Trolox E)/w (dry product) have been included.

of future health-oriented food formulations, reinforcing its role in both human health promotion and sustainable food innovation.

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## AUTHORS' CONTRIBUTIONS

Conceptualisation: H.G.-L., S.R., N.C., and É.P.-E.; Methodology: H.G.-L., S.R., N.C.; Formal Analysis: H.G.-L., S.R., N.C.; Investigation: H.G.-L., S.R., and N.C.; Writing – Original Draft Preparation: H.G.-L., S.R., and N.C.; Writing – Review & Editing: H.G.-L., N.C., and É.P.-E.; Supervision: É.P.-E.; Funding Acquisition: É.P.-E. and J.M.B. All authors have read and agreed to the published version of the manuscript.

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## CONFLICT OF INTERESTS

None.

## DATA AVAILABILITY

Data are available upon request.

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